Synthesis and Characterization of Hydroxyproline Ferrocene-Conjugated Derivatives

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Abstract

The synthesis of ferrocene-hydroxyproline amino acid conjugates was carried out using standard peptide coupling conditions in solution. The coupling of hydroxyproline (Hyp) derivatives 3 and 4 with 1-ferrocene monocarboxylic acid (Fc-COOH) (1) gave 75-90% yield of compounds 5 and 6, while the reaction with the glycine-hydroxyproline dipeptide 11 gave about 40% of conjugate 12. The reaction of 1,1′-ferrocene dicarboxylic acid (Fc(COOH)2) (2) with 3, 4 and 11 produced the corresponding Ferrocene-conjugated derivatives 7, 8 and 13 in acceptable yields (30-45%). All compounds were characterized by NMR, IR, MS and circular-dichroism spectroscopy (CD). CD-spectroscopy of compound 13 showed P-helical conformation of Fc in solution (1,2′-conformer), and for compound 7 showed no absorption which suggests the 1,3′-conformation pattern.

Keywords: Metallocene; Liquid-phase peptide coupling; Hydroxyproline; H-bonding; P-helical.

Introduction

Ferrocene (Fc) is an organometallic compound which was synthesized in 1951 by Pauson and Kealy.[1, 2] There is no individual covalent bond between the Cp ring and Fe atom, therefore a free rotation about the Cp-Fe axis was detected.[3] Substituted ferrocenes gain a great interest because of their application as bioactive substances,[4-6] material science,[7] and asymmetric catalysts. They are used as ligands in the catalysts.[8, 9] Friedel-Craft acylation of ferrocene is the most characteristic electrophilic aromatic substitution reaction. 1-acyl- and 1,1′-diacylferrocene could be prepared via acylation reaction.[10, 11] Hypohalite oxidation of the acyl group leads to preparation of 1-ferrocene carboxylic acid Fc-COOH (1) and 1,1′-ferrocene dicarboxylic acid Fc(COOH)2 (2), respectively.[12, 13] Conjugation of ferrocene with amino acids and peptides has been extensively studied.[14, 15] Compounds 1 and 2 are considered to be starting materials to prepare asymmetrical[16] and symmetrical conjugated Fc-oligopeptides.[17] The synthesis of oligopeptides is carried out in solution as well as in solid phase.

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Carbodiimides (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)[18] or N,N'-Dicyclohexylcarbodiimide (DCC)[19]) and N-Hydroxybenzotriazole (HOBt)[20] are used as coupling reagents to prepare the active ester intermediates, which lead to the desired peptide coupling.[21,22] Symmetrical Fc peptide conjugates exhibit intramolecular H-bonding between the two peptide sequences.[23] This results in the increase of the ability of the peptide side chains to assemble in specific conformation. The Fc moiety does not avoid the intermolecular H-bonding, so larger aggregates are possible to be formed.[23] H-bonding patterns in Fc(Gly-OEt)₂ and Fc(Gly-OH)₂ were studied by Hirao et. al. and Kraatz et. al. They found that the intramolecular H-bonding patterns of Fc(Gly-OEt)₂ result in the formation of 1,3'-conformer and of Fc(Gly-OH)₂ formation of 1,2'-conformer.[3, 24-26] Hirao et. al. and Kraatz et. al. have studied a series of Fc-proline dipeptides. Although the X-ray structure of Fc(L-Pro-OBzl)₂ showed a 1,3'-conformation pattern, the dipeptide Fc(L-Ala-L-Pro-OBzl)₂ and Fc(D-Ala-D-Pro-OBzl)₂ showed 1,2'-conformation. Only two strong intramolecular H-bonds between both Ala-Pro-OR sequences were observed in both Fc(L-Ala-L-Pro-OR)₂ and Fc(D-Ala-D-Pro-OR)₂.[21, 24, 27, 28] Herrik and co-workers studied Fc(COOH)₂ amino acid conjugates with Val-OMe, Phe-OMe and Pro-OMe.[16, 29] The x-ray structure of these conjugates displays two equivalent H-bonds which are formed between N-H of one amino acid proximal to Fc and the C=O of the other amino acid in both cases of Val and Phe. They gave 1,2'-conformer with 10-memberd H-bond ring (Herrick conformation), and this is similar to that of β-turn model. Additionally, in the absence of H-bonds as in the case of Pro, the 1,3'-conformer was suggested to reduce the steric effect of the Pro residue. This result was proved by Heinze et. al. using theoretical studies.[30, 31] Ferrocenes (1 and 2) and hydroxyprolines are suggested to prepare Fc-oligopeptide derivatives. 3-trans- and 4-
trans- L-Hydroxyproline derivatives (4 and 3 respectively) are considered to be good candidates for this study.

**Experimental Part**

**Materials and methods**

All reagents used were of analytical grade. 1-Ferrocene monocarboxylic acid (Fc-COOH) (1) and 1,1’-ferrocene dicarboxylic acid (Fc(COOH)2) (2) were purchased from Alfa Aesar. Solvents were dried by standard methods if necessary. Thin Layer Chromatography (TLC) was carried out on aluminum sheets precoated with silica gel 60F254 (Merck). Detection was accomplished by UV light (λ = 254 nm). Preparative column chromatography was carried out on silica gel 60 (Merck, 40 - 63 µm). 1H-NMR spectra were recorded on an AMX400 (Bruker Physik AG, Germany). Deuterated-chloroform (CDCl₃, δ = 7.26 ppm) or deuterated-dimethyl sulfoxide (DMSO, δ = 2.50 ppm) are used as internal standard, 13C-NMR spectra were calibrated with 13CDCl₃ (δ = 77.00 ppm) and DMSO (δ = 39.43 ppm) as internal standard. MS (APCI) was performed on a LCQ advantage spectrometer. HR-MS (Cl) was performed on a MAT 95XL spectrometer. Infra red spectra (IR, neat) of the products were recorded on Perkin Elmer spectrum one FT-IR spectrometer. CD-spectroscopy measurements were carried out on JASCO (J-810) spectrometer at the conditions; r.t., 0.1 cm cuvet, and sample concentration of 1.0 x 10⁻³ M in acetonitrile.

**General procedures**

**GP1. Alcohol protection.**

1 eq of hydroxyproline was dissolved in dry Dichloromethane (CH₂Cl₂). 2.1 eq of Triethylamine (TEA) was added to the solution. 1.1 eq of 4-(dimethylamino)-pyridine (DMAP) was added. The mixture was stirred at 0 °C. A solution of 1.5 eq of tert-Butyl dimethylsilyl chloride (TBS-Cl) in dry CH₂Cl₂ was added to the mixture. The mixture is left to warm up to r.t over 20h to reach the completion. The mixture was diluted with CHCl₃ and washed with sat. NH₄Cl solution. The collected organic layer was washed with sat. NaHCO₃, followed by washing with sat. NaCl. The collected organic layer was dried over anhy. Na₂SO₄. The purification of the crude product using column chromatography (SiO₂, CH₂Cl₂ / MeOH 100:2 v/v) afforded a quantitative yield of the product as yellow oil.

**GP2. Methyl ester formation**

1 eq of Hyp-OH II was dissolved in a methanolic HCl solution of ~1.25 M while stirring. The reaction was followed by TLC (Ethyl acetate). The reaction completion was after 3 days. The reaction mixture was neutralized with 0.5 M NH₃ solution and
extracted with 20 mL x 4 of CHCl₃/i-PrOH (3:1). The crude product was used without further purification.

**GP3. Peptide coupling**

Solution 1: To a solution of 1.0 eq of the free acid component (Fc-COOH (1)) in dry CH₂Cl₂, HOBr (1.0 eq) was added and cooled to 0 °C then stirred for 10 min. EDC (1.0 eq) was added to the solution and stirred for additional 30 min at 0 °C. HOBr and EDC (2.0 eq) were used in the case of the diacid Fc-(COOH)₂ (2).

Solution 2: 0.83 eq of free amine component was dissolved in dry CH₂Cl₂. TEA (1.0 eq) was added to the mixture. Solution 2 was added to solution 1 while stirring at 0 °C and left to warm up overnight. The free amine and TEA (1.66 eq) were used in the case of the diacid Fc-(COOH)₂ (2). The reaction mixture was diluted with CH₂Cl₂ and washed with 10% citric acid solution, sat. NaHCO₃ and sat. NaCl, respectively. The organic layer was dried over anhydrous Na₂SO₄. The organic solvent was removed under vacuum. The product was purified using column chromatography.

**GP4. Boc-group deprotection**

Trifluoroacetic acid (TFA) (0.5 mL) was added gradually to a mixture of a solution of 1.0 eq of the Boc-protected dipeptide in 1 mL of CH₂Cl₂ and 0.5 mL of anisole to cleave the Boc-protective group (15 min, rt). After the completion of the reaction, the mixture was diluted with 2 mL toluene and evaporated under reduced pressure to dryness. A quantitative yield was observed. The product was used without further purification.

4-trans-Hyp(OTBS) methyl ester (3)

Hyp-OMe (1) (0.5 g, 2.76 mmol) was dissolved in dry CH₂Cl₂ (15 mL). The solution was treated with TEA (0.785 mL, 5.80 mmol). DMAP (0.372 g, 3.04 mmol) was added, followed by addition of a solution of TBS-Cl (0.645 g, 4.14 mmol) in CH₂Cl₂ (5 mL). The reaction was carried out according to GP1. A quantitative yield (715 mg) of yellow oil 3 was isolated. Rf = 0.25 (CH₂Cl₂/MeOH 100:2); H-NMR (400 MHz, CDCl₃, 300 K, ppm): δ = 5.44 (bs, 1H, NH), 3.95 (bs, 2H, CH₂), 3.77 (s, 3H, OCH₃), 3.74-3.66 (m, 1H, H-α), 3.34 (dd, 1H, J = 2.3 Hz, J = 10.3 Hz, O-CH), 2.27-2.15 (m, 1H, CH₂), 2.14-2.00 (m, 1H, CH₂), 0.88 (s, 9H, t-Bu), 0.09 (s, 3H, Si-CH₃), 0.08 (s, 3H, Si-CH₃); C-NMR (100 MHz, CDCl₃, 300 K, ppm): δ = 172.4 (C=O), 70.6 (C-O), 57.8 (C-α), 54.2 (C-N), 52.4 (C-O), 43.1 (CH₂), 38.1 (CH₃), 28.3 (CH₃), 25.7 (CH₃), 17.9 (C-q), -4.81 (Si-CH₃), -4.89 (Si-CH₃); Ms (C₁₂H₂₅NO₃Si): Calculated = 260.2 (M+H), Found = 260.3 (M+H); HR-Ms: Calculated = 260.16820 (M+H), Found = 260.16800 (M+H).
3-trans-Hyp(OTBS) methyl ester (4)

Hyp-OMe (III) (0.744 g, 4.11 mmol) was dissolved in dry CH₂Cl₂ (20 mL). The solution was treated with TEA (1.20 mL, 8.25 mmol). DMAP (0.553 g, 4.52 mmol) was added, followed by addition of a solution of TBS-Cl (1.28 g, 8.22 mmol) in CH₂Cl₂ (10 mL). The reaction was carried out according to GP1. A quantitative yield (1.06 g) of the yellow oil 4 was isolated. \( R_f = 0.44 \) (CH₂Cl₂/MeOH 100:5); \( \delta = 4.52-4.42 \) (m, 1H, H-C-OSi), 3.76 (s, 3H, OCH₃), 3.70-3.64 (m, 1H, H-\( \alpha \)), 3.22-3.09 (m, 2H, HN-C₂H₅), 2.56 (bs, 1H, NH), 1.96-1.68 (m, 2H, CH₂), 0.90 (s, 9H, t-Bu), 0.10 (s, 3H, Si-CH₃), 0.10 (s, 3H, Si-CH₃); \( ^{13}C-NMR \) (100 MHz, CDCl₃, 300 K, ppm): \( \delta = 173.7 \) (C=O), 76.8 (C-OH), 68.9 (C-\( \alpha \)), 52.1 (OCH₃), 45.4 (HN-CH₂), 36.8 (CH₂), 25.6 (t-Bu-CH₃), 17.9 (C-q), -4.90 (Si-CH₃), -4.94 (Si-CH₃); Ms (C₁₂H₂₅NO₃Si): Calculated = 260.2 (M+H) (Found = 260.2 (M+H)); HR-Ms: Calculated = 260.16820 (M+H) (Found = 260.16780 (M+H)).

3-trans-Hyp methyl ester (III)

3-trans-Hyp II (2.01 g, 15.3 mmol) was dissolved in dry Methanol (44 mL). A solution of Methanolic HCl (11 mL, 3.0 M) was added while stirring to get \( \sim 1.25 \) M solution. The reaction was carried out according to GP2. An amount of (1.0 g, 45 %) of the crude product was obtained. The crude product III was used without further purification. \( R_f = 0.5 \) (Ethyl acetate); \( ^{1}H-NMR \) (400 MHz, CDCl₃, 300 K, ppm): \( \delta = 4.52-4.42 \) (m, 1H, H-C-OH), 3.83-3.66 (m, 4H, OCH₃ and H-\( \alpha \)), 3.22-3.09 (m, 2H, HN-CH₂), 2.21 (bs, 1H, NH), 2.08-1.93 (m, 1H, CH₂), 1.91-1.77 (m, 1H, CHH); \( ^{13}C-NMR \) (100 MHz, CDCl₃, 300 K, ppm): \( \delta = 173.6 \) (C=O), 75.9 (C-OH), 68.5 (C-\( \alpha \)), 52.4 (OCH₃), 44.8 (HN-CH₂), 34.6 (CH₂); Ms (C₆H₁₁NO₃): Calculated = 146.1 (M+H) (Found = 146.0 (M+H)); HR-Ms: Calculated = 146.08172 (M+H) (Found = 146.08180 (M+H)).

Fc-CO-4-trans-Hyp(OTBS)-OMe (5)

Solution 1: Fc-COOH (1) (100 mg, 0.386 mmol), HOBt (61 mg, 0.450 mmol) and EDC (82 mg, 0.425 mmol) were dissolved in CH₂Cl₂ (10 mL) according to GP3.

Solution 2: 4-trans-Hyp-(OTBS)-OMe (3) (110 mg, 0.425 mmol) was dissolved in CH₂Cl₂ (5 mL) and neutralized with TEA (59 µL, 0.425 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO₂, CH₂Cl₂/MeOH (100:1)) to get an orange solid 5 (133 mg, 73%). \( R_f = 0.51 \) CH₂Cl₂/MeOH (100:2); \( ^{1}H-NMR \) (400 MHz, CDCl₃, 300 K, ppm): \( \delta = 4.73-4.91 \) (m, 2H, Cp-CH), 4.56-4.71 (m, 2H, Cp-CH), 4.38-4.50 (m, 2H, Cp-CH and H-\( \alpha \)), 4.26-4.36 (m, 4H, Cp-CH), 3.93 (bs, 1H, O-CH), 3.77 (s, 3H, OCH₃), 2.10 (dd, 2H, J = 12.0 Hz, J = 68.4 Hz, CH₂), 1.63 (bs, 1H, CHH), 1.25 (bs, 1H, CHH), 0.88 (s, 9H, t-Bu), 0.11 (s, 3H, CH₃), 0.10 (s, 3H, CH₃); \( ^{13}C-NMR \) (100 MHz, CDCl₃, 300 K, ppm): \( \delta = 173.6 \) (C=O), 75.9 (C-OH), 68.5 (C-\( \alpha \)), 52.4 (OCH₃), 44.8 (HN-CH₂), 34.6 (CH₂); Ms (C₆H₁₁NO₃): Calculated = 146.1 (M+H) (Found = 146.0 (M+H)); HR-Ms: Calculated = 146.08172 (M+H) (Found = 146.08180 (M+H)).
173.1 (Fc-C=O), 169.0 (C=O), 71.3 (Cp-C), 71.0 (Cp-C), 70.8 (Cp-C), 70.6 (Cp-C), 70.2 (Cp-C), 70.1 (Cp-C), 69.8 (Cp-C), 59.0 (C-\alpha), 58.7 9\text{C-O}), 52.3 (C-O), 37.6 (Hyp-C), 25.8 (2 x Hyp-C), 25.7 (C-q), 18.0 (3 x CH_3), -4.71 (Si-CH_3), -4.75 (Si-CH_3); IR-neat (cm\(^{-1}\)):
3108.9, 2929.8, 2857.0, 1764.8, 1600.9, 1169.3; Ms (C\(_{23}\)H\(_{33}\)FeNO\(_4\)Si):
Calculated = 471.2 (Found = 471.8); HR-Ms:
Calculated = 471.15283 (Found = 471.15410).

Fc-CO-trans-Hyp(OTBS)-OMe (6)
Solution 1: Fc-COOH (1) (20 mg, 0.077 mmol), HOBt (12 mg, 0.09 mmol) and EDC (17 mg, 0.09 mmol) were dissolved in CH\(_2\)Cl\(_2\) (3 mL) according to GP3.
Solution 2: 3-trans-Hyp-(OTBS)-OMe (4) (23 mg, 0.09 mmol) was dissolved in CH\(_2\)Cl\(_2\) (2 mL) and neutralized TEA with (13 \(\mu\)L, 0.09 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH (100:1)) to get the orange solid 6 (35 mg, 96%).

R_f = 0.61 CH\(_2\)Cl\(_2\)/MeOH (100:2); \(^1\)H-NMR (400 MHz, CDCl\(_3\), 300 K, ppm): \(\delta = 4.98-4.72 \text{ (m, 2H, Fc)}, 4.54 \text{ (bs, 1H, HC-OSi)}, 4.40 \text{ (bs, 3H, Fc)}, 4.28 \text{ (bs, 5H, Fc and CH)}, 4.18-3.87 \text{ (m, 2H, CH and H-\alpha)}, 3.79 \text{ (s, 3H, OCH}_3\), 2.33-2.15 \text{ (m, 1H, CHH)}, 2.11-1.96 \text{ (m, 1H, CHH)}, 0.91 \text{ (s, 9H, t-Bu)}, 0.14 \text{ (s, 3H, Si-CH}_3\), 0.13 \text{ (s, 3H, Si-CH}_3\); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\), 300 K, ppm): \(\delta = 171.4 \text{ (Fc-C=O)}, 165.2 \text{ (C=O)}, 73.8 \text{ (C-OSi)}, 70.7 \text{ (Cp)}, 70.6 \text{ (Cp)}, 69.6 \text{ (C-\alpha)}, 52.3 \text{ (OCH}_3\), 46.0 \text{ (HN-CH}_2\), 34.5 \text{ (CH}_2\), 25.6 \text{ (t-Bu-CH}_3\), 18.0 \text{ (C-q)}, -4.9 \text{ (Si-CH}_3\), -5.00 \text{ (Si-CH}_3\); IR-neat (cm\(^{-1}\)):
3087.7, 2954.1, 2930.2, 2857.2, 1744.7, 1671.7, 1602.8, 1106.0; Ms (C\(_{23}\)H\(_{33}\)FeNO\(_4\)Si):
Calculated = 471.2 (Found = 471.2); HR-Ms: Calculated = 471.15283 (Found = 471.15320).

Fc(CO-4-trans-Hyp(OTBS)-OMe)\(_2\) (7)
Solution 1: Fc(COOH)_2 (2) (50 mg, 0.165 mmol), HOBt (50 mg, 0.363 mmol) and EDC (70 mg, 0.363 mmol) were dissolved in CH\(_2\)Cl\(_2\) (10 mL) according to GP3.
Solution 2: 4-trans-Hyp-(OTBS)-OMe (3) (94 mg, 0.363 mmol) was dissolved in CH\(_2\)Cl\(_2\) (5 mL) and neutralized with TEA (50 \(\mu\)L, 0.363 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH (100:1→100:2)) to get the orange solid 7 (43 mg, 35%).

R_f = 0.19 CH\(_2\)Cl\(_2\)/MeOH (100:2); \(^1\)H-NMR (400 MHz, CDCl\(_3\), 300 K, ppm): \(\delta = 4.85 \text{ (bs, 3H, Cp-CH)}, 4.72 \text{ (bs, 2H, Cp-CH)}, 4.62-4.43 \text{ (m, 5H, Cp-CH and H-\alpha)}, 3.98 \text{ (bs, 2H, O-CH)}, 3.80 \text{ (s, 6H, 2 x OCH}_3\), 2.35-1.92 \text{ (m, 4H, 2 x CH}_2\), 1.72 \text{ (bs, 2H, CH}_2\), 1.45-1.14 \text{ (m, 2H, CH}_2\), 0.88 \text{ (s, 18H, 2 x t-Bu)}, 0.10 \text{ (s, 6H, 2 x CH}_3\), 0.08 \text{ (s, 3H, CH}_3\), 0.07 \text{ (s, 3H, CH}_3\); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\), 300 K, ppm): \(\delta = 172.9 \text{ (Fc-C=O)}, 169.2 \text{ (C=O)}, 72.9 \text{ (Cp-C)}, 72.7 \text{ (Cp-C)}, 72.4 \text{ (Cp-C)}, 71.2 \text{ (Cp-C)}, 71.0 \text{ (Cp-C)}, 58.9 \text{ (C-\alpha)}, 56.9 \text{ (C-O)}, 52.2 \text{ (C-O)}, 37.7 \text{ (Hyp-C)}, 25.7 \text{ (2 x Hyp-C)}, 25.6 \text{ (C-q)}, 17.9 \text{ (3 x CH}_3\).
CH$_3$), 0.98 (Si-CH$_3$), -4.79 (Si-CH$_3$), -4.91 (Si-CH$_3$); IR-neat (cm$^{-1}$): 3108.9 , 2929.5 , 2856.9, 1732.6, 1600.5, 1022.3; Ms (C$_{36}$H$_{56}$FeN$_2$O$_8$Si$_2$): Calculated = 756.3 (Found = 756.3); HR-Ms: Calculated = 756.29246 (Found = 756.29380).

Fc(CO-3-trans-Hyp(OTBS)-OMe)$_2$ (8)

Solution 1: Fc(COOH)$_2$ (2) (23 mg, 0.076 mmol), HOBt (25 mg, 0.182 mmol) and EDC (35 mg, 0.182 mmol) were dissolved in CH$_2$Cl$_2$ (5 mL) according to GP3.

Solution 2: 3-trans-Hyp-(OTBS)-OMe (4) (43 mg, 0.166 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and neutralized with (25 µL, 0.182 mmol) of TEA. Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH (100:1→100:2)) to get the orange solid 8 (25 mg, 43 %). $R_t = 0.30$ CH$_2$Cl$_2$/MeOH (100:2); $^1$H-NMR (400 MHz, CDCl$_3$, 300 K, ppm): $\delta = 5.09$-4.77 (m, 5H), 4.72-4.28 (m, 11H), 3.79 (s, 6H), 2.35-2.15 (m, 2H), 2.10-1.93 (m, 2H), 0.91 (s, 18H, 2 x t-Bu), 0.14 (s, 6H, 2 x Si-CH$_3$), 0.13 (s, 6H, 2 x Si-CH$_3$); $^{13}$C-NMR (100 MHz, CDCl$_3$, 300 K, ppm): $\delta = 171.3, 171.2, 171.1, 168.5$ (C=O), 73.3 (C-OSi), 73.0 (C-OSi), 72.7 (Cp), 71.8 (Cp), 71.7 (Cp), 69.2 (C-α), 69.1 (C-α), 52.3 (OCH$_3$), 46.1 (N-CH$_2$), 34.6 (CH$_2$), 25.7 (t-Bu-CH$_3$), 18.0 (C-q), -4.9 (Si-CH$_3$), -5.00 (Si-CH$_3$); IR-neat (cm$^{-1}$): 3087.5, 2952.6, 2929.3, 2856.7, 1744.3, 1671.5, 1609.8, 1173.6; Ms (C$_{36}$H$_{56}$FeN$_2$O$_8$Si$_2$): Calculated = 756.3 (Found = 756.2); HR-Ms: Calculated = 756.29246 (Found = 756.29380).

Boc-Gly-4-trans-Hyp(OTBS)-OMe (10)

Solution 1: Boc-Gly-OH (7) (162 mg, 0.930 mmol), HOBt (156 mg, 0.930 mmol) and EDC (178 mg, 0.930 mmol) were dissolved in CH$_2$Cl$_2$ (20 mL) according to GP3.

Solution 2: 4-trans-Hyp-(OTBS)-OMe (3) (200 mg, 0.770 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and neutralized with TEA (214 µL, 1.54 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH (100:1)) to get the colorless oily substance 10 (290 mg, 90 %). $R_t = 0.40$ CH$_2$Cl$_2$/MeOH (100:2); $^1$H-NMR (400 MHz, CDCl$_3$, 300 K, ppm): $\delta = 5.42$ (bs, 1H, CONH$_2$), 4.46-4.48 (m, 2H, Gly-CH$_2$), 3.92 (bs, 2H, CH$_2$), 3.74 (s, 3H, OCH$_3$), 3.69 (m, 1H, H-α), 3.32 (dd, 1H, $J = 2.6$ Hz, $J = 10.1$ Hz, O-CH$_2$), 2.28-2.12 (m, 1H, CH$_2$), 1.20-1.25 (m, 1H, CH$_2$), 1.44 (s, 9H, Boc-t-Bu), 0.86 (s, 9H, t-Bu), 0.07 (s, 3H, Si-CH$_3$), 0.06 (s, 3H, Si-CH$_3$); $^{13}$C-NMR (100 MHz, CDCl$_3$, 300 K, ppm): $\delta = 172.4$ (C=O), 170.4 (C=O), 167.5 (C=O), 70.6 (C=O), 77.2 (C=O), 57.8 (C-α), 54.2 (C-N), 52.4 (C=O), 43.1 (CH$_3$), 38.1 (CH$_3$), 28.7 (CH$_3$), 28.3 (CH$_3$), 25.7 (CH$_3$), 17.9 (C-q), -4.81 (Si-CH$_3$), -4.89 (Si-CH$_3$); IR-neat (cm$^{-1}$): 2954.6, 2857.9, 1750.7, 1710.4, 1659.4, 1166.5; Ms (C$_{36}$H$_{56}$FeN$_2$O$_8$Si$_2$): Calculated = 756.3 (Found = 756.2); HR-Ms: Calculated = 756.24180 (M+H) (Found = 756.24209 (M+H)).
H-Gly-4-trans-Hyp(OTBS)-OMe (11)

dipeptide 8 (195 mg, 0.47 mmol) was dissolved in CH₂Cl₂ (1 mL) and treated with TFA (0.5 mL) according to GP4. A quantitative yield of the free amine dipeptide 11 (145 mg) was obtained. The crude product was used in the next step without further purification. Rᵣ = 0.00 CH₂Cl₂/MeOH (100:2); ¹H-NMR (400 MHz, CDCl₃, 300 K, ppm): δ = 8.01 (bs, 2H, NH₂), 4.63-4.49 (m, 2H, Gly-CH₂), 4.00-3.82 (m, 2H, CH₂), 3.80-3.75 (m, 1H, H-α), 3.72 (s, 3H, OCH₃), 3.32 (dd, 1H, J = 2.0 Hz, J = 9.9 Hz, O-CH), 2.29-2.17 (m, 1H, CH₂), 2.15-2.04 (m, 1H, CH₂), 0.88 (s, 9H, t-Bu), 0.10 (s, 3H, Si-CH₃), 0.09 (s, 3H, Si-CH₃); Ms (C₁₄H₂₈N₂O₄Si): Calculated = 355.1 (M+K) (Found = 355.1 (M+K)).

Fc-CO-Gly-4-trans-Hyp(OTBS)-OMe (12)

Solution 1: Fc-COOH (1) (50 mg, 0.193 mmol), HOBt (31 mg, 0.225 mmol) and EDC (41 mg, 0.213 mmol) were dissolved in CH₂Cl₂ (5 mL) according to GP3.

Solution 2: H-Gly-4-trans-Hyp-(OTBS)-OMe (9) (67 mg, 0.213 mmol) was dissolved in CH₂Cl₂ (5 mL) and neutralized with TEA (30 µL, 0.213 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO₂, CH₂Cl₂/MeOH (100:1)) to get the orange solid 12 (40 mg, 39%). Rᵣ = 0.43 CH₂Cl₂/MeOH (100:2); ¹H-NMR (400 MHz, CDCl₃, 300 K, ppm): δ = 6.70 (t, 1H, J = 3.6 Hz), 4.74-4.70 (m, 2H), 4.66-4.52 (m, 2H), 4.37-4.33 (m, 2H), 4.25-4.20 (m, 5H), 4.19-4.17 (m, 2H), 3.85-3.66 (m, 5H), 3.42 (dd, 1H, J = 10.3 Hz), 2.31-2.20 (m, 1H), 2.13-2.02 (m, 1H), 0.88 (s, 9H, t-Bu), 0.10 (s, 6H, 2xCH₃); IR-neat (cm⁻¹): 3085.9, 2929.4, 2856.6, 1743.9, 1199.0; Ms (C₂₅H₃₆FeN₂O₅Si): Calculated = 528.2 (Found = 528.9); HR-Ms: Calculated = 528.17429 (Found = 528.17550).

Fc(CO-Gly-4-trans-Hyp(OTBS)-OMe)₂ (13)

Solution 1: Fcc(OOH)₂ (2) (50 mg, 0.165 mmol), HOBt (50 mg, 0.363 mmol) and EDC (70 mg, 0.363 mmol) were dissolved in CH₂Cl₂ (5 mL) according to GP3.

Solution 2: H-Gly-4-trans-Hyp-(OTBS)-OMe (9) (115 mg, 0.363 mmol) was dissolved in CH₂Cl₂ (5 mL) and neutralized with TEA (50 µL, 0.363 mmol). Solution 2 was added to solution 1 according to GP3. The crude product was purified using column chromatography (SiO₂, CH₂Cl₂/MeOH (100:1→100:2)) to get the orange solid 13 (43 mg, 30%). Rᵣ = 0.15 CH₂Cl₂/MeOH (100:2); ¹H-NMR (400 MHz, CDCl₃, 300 K, ppm): δ = 8.11 (d, 1H, J = 8.6Hz) ppm 7.46 (m, 1H), 5.71-4.14 (m, 16H), 4.06-3.14 (m, 10H), 2.41-1.89 (m, 4H), 1.04-0.71 (m, 18H, t-Bu), 0.28-0.01 (m, 12H, 4 x CH₃); IR-neat (cm⁻¹): 3087.7, 2952.2, 2856.7, 1743.9, 1199.0; Ms (C₄₀H₆₂FeN₄O₁₀Si₂): Calculated = 870.3 (Found = 870.8); HR-Ms: Calculated = 870.33539 (Found = 870.33610).
Results and Discussion

The hydroxyproline derivatives 3 and 4 were prepared from precursors I and III, which are treated with TBS-Cl in the presence of equimolar amount of DMAP as a base.

![Scheme 1](image)

Quantitative yields were obtained in both reactions (Scheme 1).

EDC and HOBt were used as coupling reagents. They were added to Fc-COOH (1) and Fc(COOH)₂ (2) to prepare the active ester intermediate. The hydroxyproline derivative was treated with equimolar amount of TEA to get the free amine group. The free amine solution was added to the active ester solution to get the coupling product. Coupling of compound 1 with amino acid derivatives 3 and 4 gave 73% (5) and 96% (6) yield, respectively.

![Scheme 2](image)

Whereas, coupling of dicarboxylic acid 2 with the free amines 3 and 4 afforded the ferrocene derivatives 7 and 8, respectively in acceptable yields (35-45%). The low yields are probably due to possible steric factors.
Compounds 5-8 were characterized using FT-IR, $^1$H-NMR, $^{13}$C-NMR and HR-MS spectroscopy. The $^1$H-NMR spectra of all compounds show broadening in the peaks (Figure 1). This may be due to the flexibility around Cp(centroud)-Fe-Cp(centroud), which leads to several conformers in the NMR solution.

![Figure 1: The $^1$H-NMR of compounds 6 and 8](image)

The dipeptide 10 was synthesized according to the general procedure GP3, with 90% yield, (Scheme 3). The dipeptide 10 is treated with TFA to remove the protecting Boc-group and generate the free amine 11, which is subsequently used to prepare the dipeptide conjugates 12 and 13 (Scheme 4). Dipeptide 11 was used without further purification because of its high polarity.

![Scheme 3](image)
Ferrocene derivatives 12 and 13 were obtained and purified by column chromatography using SiO\textsubscript{2} and CH\textsubscript{2}Cl\textsubscript{2}/MeOH (100:1) as solvent mixture. The products were obtained as orange solids with 39% and 30% yields, respectively. Intermediates and products 10-13 were characterized using different spectroscopic methods; HR-MS, FT-IR, \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR. Circular dichroism (CD) measurements were recorded in order to elucidate the conformations of the Fc-peptide conjugates 12 and 13.
The proton of the N-H group of compound 12 was observed as a triplet at 6.70 ppm with coupling constant of 3.6 Hz. This indicates the absence of the intramolecular H-bonding (Figure 3). The intramolecular H-bonding effect is clearly observed in the $^1$H-NMR of compound 13. The N-H proton peaks are shifted downfield to the range between 8.11 ppm with coupling constant of about 8.6 Hz. The Intramolecular H-bonding in 13 indicates that the p-helical ferrocene conformation of the peptide chains about Fc as a possible dominant conformer (Figure 3).

**Figure 2:** The $^1$H-NMR of compounds 12 and 13

**Figure 3:** The presence of intramolecular H-bonding in 13 while absent in 12.
In the amide range, several N-H peaks were observed (Figure 2); this could indicate several conformers and aggregates or could refer to some byproducts (OBt-derivatives) of the coupling process (Figure 4).\textsuperscript{[20, 33, 34]}

![Figure 4: The possible byproducts (OBt-derivatives).](image)

The helical chirality of the ferrocene (Fc) group in solution has been monitored by circular dichrosim (CD) spectroscopy.\textsuperscript{[32]} As expected monosubstituted ferrocene 12 does not show a CD band in the ferrocene region (400-600 nm), while the disubstituted ferrocene 13 shows a moderate absorption CD band in the ferrocene region.

![Figure 5: The CD-spectra of both compounds 12 and 13 in acetonitrile.](image)
This result indicates an ordered p-helical ferrocene conformation of the peptide chains about Fc as dominant conformer. Interestingly, the presence of the flexible glycine residue in close proximity to Fc center assists such conformation (1,2'-conformer). This result is supported by comparing the CD spectra of compounds 12 and 13 (Figure 5).

![CD spectrum](image)

**Figure 6:** The circular-dichroism spectra of compounds 7 in acetonitrile.

The absence of the glycine residue as in the disubstituted ferrocene 7 shows very week absorption in the CD spectrum represented in figure 4. This indicates the 1,3'-conformation of compound 7 as a dominant conformer (Figure 6).

**Conclusion**

In this work, hydroxyproline derivatives were coupled with 1-ferrocene monocarboxylic acid Fc-COOH and 1,1'-ferrocene dicarboxylic acid in order to prepare ferrocene-conjugated peptides in very good yields to acceptable ones. The conjugates were characterized by several spectroscopic methods including NMR, IR, HR-MS, and circular dichroism (CD). The CD measurements show two suggested conformers (Herrick conformation) of disubstituted ferrocene conjugates. In the presence of H-bonding, the 1,2'-conformer is the dominant, while the 1,3'-conformer is dominant in the absence of H-bonding to decrease the steric effect.
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References